Multiomic spatial interrogation of tumor-infiltrated immune cells using the RNAscope™ Co-detection assay and the new TSA Vivid dyes

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INTRODUCTION

Interrogating complex tumor microenvironment requires a multi-omics approach that can provide high level of sensitivity and specificity. Identifying immune cell subsets within the tumor can be vital for predicting response and determining therapeutic efficacy. Here, we demonstrate a newly developed integrated ISH and IHC/IF (immunohistochemistry/ immunofluorescence) workflow compatible with manual and automated platforms that can substantially improve RNA-protein co-detection.

We demonstrate the use of our RNA-Protein Co-detection assay in combination with the automated and manual RNAscope Multiplex Fluorescent v2 assay and the RNAscope Chromogenic Duplex assay. The RNAscope Multiplex Fluorescent v2 assay was also combined with the new TSA Vivid dyes for detection of cytokines and immune cells. Here, we demonstrate the utility of the co-detection assays in detecting T cell markers, macrophage markers and checkpoint markers in the tumor microenvironment by using a tumor microarray.

Overall, the new RNAscope-ISH-IHC co-detection workflow and reagents enable optimized simultaneous visualization of RNA and protein targets by enhancing the compatibility of antibodies and requiring minimal optimization.

METHODS

RNAscope integrated co-detection workflow for simultaneous detection of RNA and protein biomarkers

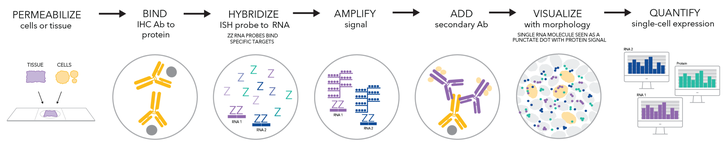

Figure 1: RNAscope ISH-IHC integrated co-detection workflow for detecting RNA and protein targets on the same section of the tissue

❑ ***Tissues: Tumor Tissue Microarray***

❑ ***Probe and antibodies used for detecting immune cells, tumor cells and cytokine markers***

Combinations	RNA probe	RNA probe	RNA probe	Antibody	Assay Platform
Combo 1	*PanCK*	*PD-1*	*CTLA4*	PD-L1	Multiplex Fluorescent, Manual
Combo 2	*GZMB*	*IFNG*	-	CD8	Multiplex Fluorescent, Automated
Combo 3	*CD163*	*ITGAM*	-	CD68	Multiplex Fluorescent, Automated
Combo 4	*TNFA*	-	-	CD3	Chromogenic Duplex, Automated
Combo 5	*CCL5*	*NOS2*	-	CD8	Chromogenic Duplex, Automated
Combo 6	*TNFA*	*IFNG*		CD3	Multiplex Fluorescent with new Fluorescent Vivid dyes, Automated

RESULTS

PD-L1 was predominantly expressed in the stromal region of breast cancer tissue but it was co-expressed with cytokeratin in tumor cells of lung cancer tissue

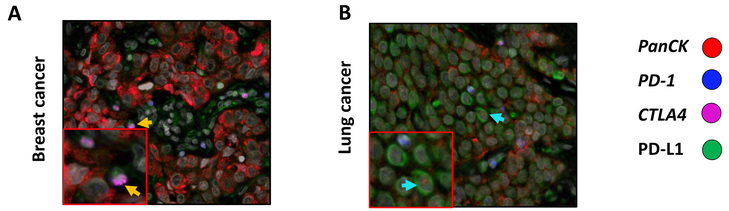

Figure 2: Manual RNAscope ISH-IF co-detection assay to visualize immunoregulatory markers in tumor tissues. A and B, expression of *PanCK*, *PD-1* and *CTLA4* was detected by RNAscope ISH while expression of PD-L1 was detected using IF. PD-L1+/*CTLA4*+ cells (🔥) , PD-L1+/*PanCK* + cells (💧)

Tumor infiltrating lymphocytes and macrophages were detected using fluorescent and chromogenic automted assays

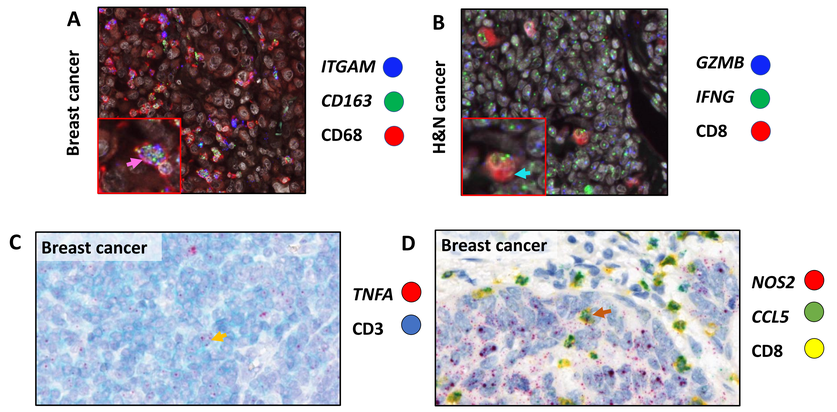

Figure 3: Automated RNAscope ISH-IF and ISH-IHC co-detection assays to detect T cells and macrophages. A, *ITGAM* and *CD163* were detected using RNAscope ISH and CD68 was detected using IF in breast cancer tissue, **B** , *GZMB* and *IFNG* were detected using RNAscope ISH and CD8 was detected using IF in head and neck cancer tissue, **C**, expression of *TNFA* was visualized by RNAscope ISH and CD3 was detected using IHC, **D,** expression of *NOS2* and *CCL5* was visualized by RNAscope ISH while CD8 was detected using IHC. *ITGAM*+/*CD163*+/CD68+ (💧), *GZMB*+/*IFNG*+/CD8+ (💧), CD3+/*TNFA*+ T cells (🔥), CD8+/*CCL5*+ T cells (🔥)

New RNAscope Multiplex v2 assay with TSA Vivid dyes improves sensitivity of cytokine detection in tumor tissues

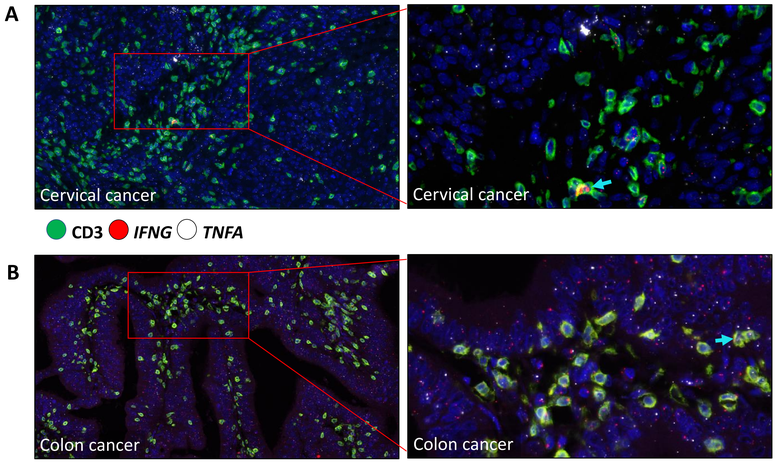

Figure 4: Automated RNAscope ISH-IF using TSA Vivid dyes to visualize T cells and cytokines. Expression of *TNFA* and *IFNG* was visualized by RNAscope ISH and CD3 was detected using IF in **A,** cervical cancer and **B,** colon cancer. *IFNG*/*TNFA*/CD3+ (💧)

SUMMARY

- Using the RNAscope RNA protein co-detection assays we assessed the tumor microenvironment and characterized tumor infiltrated immune cells.
- We identified immune cell sub-populations using cell marker-specific antibodies and detected expression of activating chemokines and cytokines using RNAscope ISH.
- With the new Multiplex Fluorescent v2 assay with Vivid dyes, low-abundance cytokines were visualized with increased signal intensity and resolution.
- The co-detection reagents and workflow are compatible with **manual and automated platforms** and can be combined with most RNAscope, BaseScope and miRNAscope assays.

CONCLUSION

The new **RNA-protein Co-detection workflow** improves IHC/IF antibody compatibility with the RNAscope ISH technology creating an extremely powerful multiomic solution to elucidate cellular heterogeneity, identify novel cell populations while retaining spatial information with morphological context.

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